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Arbovirus-Related Encephalitis

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Arthropod-borne virus encephalitis in the U.S.A. includes LaCrosse, St Louis, western equine, eastern equine, Venezuelan equine, and Powassan in that order of frequency. Diagnosis can be aided by the history of seasonal occurrence, climate, geographic location, exposure to vectors, and age of the patient. The definitive diagnosis is usually made by serological tests such as neutralization, complement-fixation, hemagglutination-inhibition, and immunofluorescence; the radioimmune assay and the enzyme-linked immunosorbent assay show promise of future utility.

These diseases are prevented by vector control. It is unlikely that vaccines or anti-viral agents will have application in the near future.

Encephalitis is a reportable disease in the United States. The arboviruses comprise 65 percent of encephalitis cases reported by etiology to the Center for Disease Control over the past 10 years. Nearly all are mosquito-borne and caused by five viruses: LaCrosse, St. Louis encephalitis, western equine encephalomyelitis, eastern equine encephalitis, and Venezuelan equine encephalomyelitis, in that order of frequency. Ten cases of Powassan encephalitis, a tick-borne viral disease, have also been recorded in the U.S.A. as sporadic cases since the virus was first isolated (in Canada) in 1958. Table 1 shows the number of reported cases of arthropod-borne encephalitis over an eight-year period. In addition, about 60 percent of reported encephalitis is never diagnosed as to etiology; most of these cases occur in the late summer and early fall when mosquitoes are prevalent.

A high index of suspicion is needed by the physician and the laboratory diagnostician if cases of arboviral encephalitis are to be recognized. Although in an epidemic it may be possible to recognize a diagnostic clinical syndrome, isolated cases are not usually diagnosed reliably without specific serologic or virologic tests. Suspicion is guided best by a knowledge of epidemiologic factors which in turn depends on knowledge of the specific vector of each virus, the geographic distribution, and on the mode of reservoir maintenance. The following descriptions will summarize what the clinical virologist needs to know to maintain appropriate index of suspicion and to pursue a rational approach to diagnosis of arboviral encephalitis. For a detailed discussion of differential diagnosis and a comparison with other types of CNS disease the reader should consult the review by Monath [1].

EPIDEMIOLOGY

LaCrosse virus is transmitted by *Aedes triseriatus* mosquitoes, a woodland species widely distributed in the middle western states as well as the middle Atlantic and Appalachian states. The virus is transmitted vertically through the mosquito egg [2], and the adult progeny can transmit the virus by bite on emergence from the pupal stage. This type of cycle allows for human La Crosse virus infection in early summer, unlike infection with western equine encephalitis (WEE) or St. Louis encephalitis

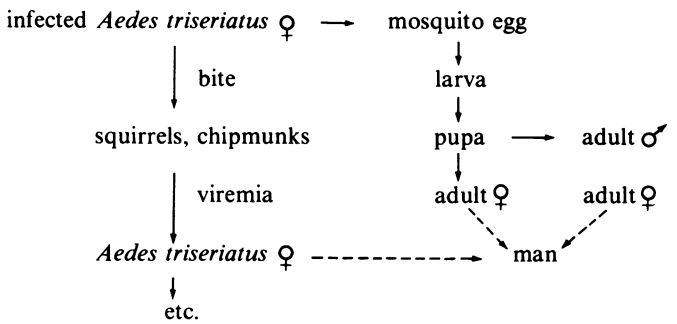
TABLE 1
Reported Cases of Arthropod-Borne Encephalitis, 1969–1976*

	WEE	EEE	SLE	CE	VEE	POW	TOTAL
1969	21	3	16	67	1	0	108
1970	4	2	15	89	0	0	110
1971	11	4	57	58	19	1	150
1972	8	0	13	46	2	1	70
1973	4	7	5	75	0	0	91
1974	2	4	72	30	0	0	108
1975	133	3	2131	160	2	2	2431
1976	1	0	376	31	0	0	408

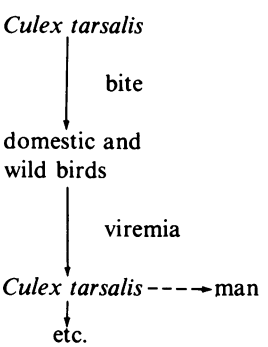
*Data from Viral Diseases Division, CDC, Atlanta, Georgia

(SLE) which appear late in the summer. The transmission cycle is amplified by infection and subsequent viremia in woodland mammals, especially in chipmunks and tree squirrels [3]. The cycle probably also is maintained by venereal transmission in mosquitoes through the infected fluid of the seminal vesicles [4]. In the laboratory as many as 30 percent of infected male mosquitoes transmit virus this way.

The disease affects mainly children. Man is infected when exposed to mosquitoes in wooded areas, either by living in a suburban community nestled among hardwood forests, or by hunting, fishing, picnicking, or hiking in the woods. Back yard tire swings and discarded tires offer breeding sites for *Aedes triseriatus* mosquitoes and serve as foci of infection. The LaCrosse virus transmission cycle is as follows:



St. Louis encephalitis occurs endemically in irrigated areas of California, Texas, and probably in other parts of south central and southwestern U.S.A. where *Culex tarsalis* mosquitoes abound. SLE accounts for sporadic cases and occasional small outbreaks of human encephalitis each year in these endemic areas. The endemic cycle is as follows:



SLE virus requires warm weather, probably many days over 90° F [5], to complete its extrinsic incubation (in the mosquito) effectively. A wide variety of birds are involved in the transmission cycle, including sparrows, pigeons, and blackbirds. The mosquito, *Cu. tarsalis*, breeds in ground pools and therefore thrives in irrigated fields and in years of wet weather in other regions. The mosquito is uncommon in eastern U.S.A. The overwintering mechanism of SLE virus is not known. Theories include transovarial transmission, persistent infection in birds, in bats, in snakes, or in hibernating adult mosquitoes [6].

Epidemic St. Louis encephalitis is the most devastating encephalitic disease in the U.S.A. The epidemic vector is *Culex pipiens*, which occurs as the *quinquefasciatus* subspecies in the South and as the *pipiens* subspecies in the North. The mosquito breeds in sewage or other water with high organic content found in the major cities of the U.S. The epidemics of SLE are thus urban or suburban. Paradoxically SLE epidemics occur in drought years because *Culex pipiens* are most abundant in poorly draining sewage. The year of the first major epidemic (1933) in St. Louis was the driest year since 1837 when records were first kept [7]. The SLE epidemic cycle is shown schematically as follows:

Culex pipiens (complex)

↓ bite

domestic and wild birds

↓ viremia

Culex pipiens ------→ man

↓

etc.

In Florida, *Culex nigripalpus* replaces *Culex pipiens quinquefasciatus* as the urban vector.

Western equine encephalomyelitis is transmitted by *Culex tarsalis* mosquitoes with a cycle much like the endemic transmission of SLE virus. In the southwest the two forms of encephalitis are seen together. WEE virus, however, differs in that it is able to replicate in the mosquito at much cooler temperatures and thus causes epidemic disease earlier in the summer, and eventually much farther north (into Canada) later in the season.

Eastern equine encephalitis is more commonly observed in horses and pheasants than in man. The virus is transmitted among birds by *Culiseta melanura*, a swamp mosquito in which it appears annually and is maintained by an unknown mechanism. The infection is found along the Atlantic and Gulf coasts as well as in the lake region of New York State. Transmission during epidemics may be by *Aedes* mosquitoes. The cycle is as follows:

Culiseta melanura

↓ bite

birds ⟶ *Aedes* ⇢ horses

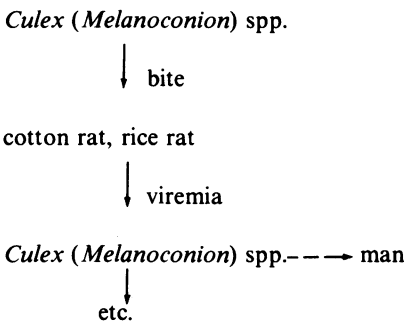
↓ viremia ⇢ man

Culiseta melanura

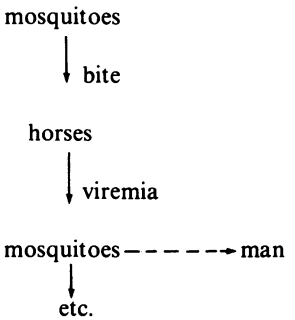
↓

etc.

Venezuelan equine encephalomyelitis is endemic in the Florida Everglades where sporadic human Venezuelan encephalitis occurs but is a rare disease [8]. *Culex* (*Melanoconion*) spp. mosquitoes maintain the cycle in the swamp, transmitting to small rodents. Man is infected only when he lives in the swamp or enters for recreation [8]. The endemic cycle is as follows:



An epidemic of VEE occured in 1971 when a distinct VEE serotype which had previously caused epidemic disease in Middle America was introduced to the U.S.A. through Mexico. The cycle involved a variety of mosquitoes and horses as follows:



The epidemic was controlled by vaccination of horses and by spraying to kill mosquitoes. The epidemic subtype of the virus was apparently eradicated from Texas and has not been detected in the U.S.A. since.

The physician must consider not only the season, climate, geographic distribution, and exposure of the encephalitic patient to vectors, but also the age of the patient. LaCrosse encephalitis is a disease of children; the clinical attack rate and case fatality rate of SLE is much greater in older persons; WEE has a higher attack rate and severity in infants; EEE is a severe disease in children although it also causes encephalitis in other age groups.

The major outbreaks of SLE and WEE during the past decade are shown in Table 2. Cases of LaCrosse encephalitis occur at a relatively constant rate each year although the disease is being recognized now much more frequently in some parts of the U.S.A., such as New York State, than previously.

LABORATORY DIAGNOSIS

Diagnosis of arboviral encephalitis is generally made by serologic tests of acute and convalescent sera. A fourfold or greater rise or fall in antibody titer is diagnostic of infection. The tests commonly used are hemagglutination-inhibition (HI),

TABLE 2
Epidemics of Arboviral Encephalitis in the U.S.A., 1969–1978

1974	St. Louis encephalitis	Tennessee & Mississippi	47 cases
1975	western encephalitis	Red River Valley	84 cases
1975	St. Louis encephalitis	central & eastern U.S.A.	2,131 cases
1977	St. Louis encephalitis	Florida	51 cases

complement-fixation, neutralization, and fluorescent antibody. IgM appears during the first two weeks of illness, is type-specific, and is measured by the HI and neutralization tests. IgG measurable by all four tests appears usually after the second week of illness. As a rule, the neutralization reaction is most specific, the CF reaction less so, and the HI reaction the least specific.

These serologic reactions (including the HI test) in the U.S.A. are generally relatively specific, unlike those in the tropics where heterologous reactions with closely related viruses may complicate interpretation of the serologic response. In the U.S.A., the diagnosis of St. Louis encephalitis when the disease occurs in persons with preexisting dengue antibody may be impossible to make because of the characteristic broad flavivirus group anamnestic response [9]. Additionally, La-Crosse virus is so closely related to several other members of the California group of viruses that a specific diagnosis may not be possible on a serologic basis [10]. These examples of difficult diagnostic situations are exceptions rather than the rule. The neutralization and HI tests are reliable and are preferred over the CF test which does not become positive in some patients.

Rarely, a virus may be isolated directly from blood, but in most patients, by the time encephalitis is recognized the viremic phase of the infection is past. Virus is almost never found in the cerebrospinal fluid. At post mortem if death occurs during the first three or four days of illness, the virus may be recovered by inoculation of brain suspensions intracerebrally into baby mice or into tissue cultures.

RECENT ADVANCES

Recent advances are most notable in the area of epidemiology of encephalitogenic arboviruses. LaCrosse virus was shown in 1973 by workers at the University of Wisconsin to be transmitted transovarially both in the laboratory [2] and in its natural habitat [11] by *Aedes triseriatus*. This was the first demonstration of vertical transmission of a mosquito-borne virus. Although the dynamics of transovarial transmission may be insufficient to explain the complete maintenance of the virus as claimed by some [12], amplification in tree squirrels and chipmunks [3] as well as venereal transmission in the mosquito [4] offer excellent auxiliary mechanisms.

These observations of transovarial transmission and observations of French scientists of a similar mechanism in Africa for flaviviruses [13] (the family of arboviruses responsible for yellow fever, St. Louis encephalitis, and Japanese encephalitis) stimulated the research of Rosen et al. [14] of transovarial transmission of Japanese encephalitis virus, a pathogen closely related to St. Louis encephalitis virus. It is now clear that transovarial transmission is a characteristic of many mosquito-borne flaviviruses [15] although only a very small number of progeny are infected, and thus its real significance in nature is yet to be demonstrated. At the same time *Culex pipiens* mosquitoes naturally infected with St. Louis encephalitis virus were found alive and well during February in Pennsylvania and Maryland [16]. This important observation offers another viable alternative for the overwintering mechanism of St. Louis encephalitis virus.

We still have virtually no clues as to the mechanism of overwintering of alphaviruses (the genus of arboviruses causing eastern, western, and Venezuelan encephalomyelitis). New and more sensitive techniques for isolating arboviruses may provide the answers to the question of where the viruses hide out during times of low or no transmission. The baby mouse historically was used to isolate arboviruses; it was recently found that laboratory-raised mosquitoes [17] were more sensitive than mice for primary isolation, and now the development of the C6/36 clone of the *Aedes albopictus* (Singh) cell line [18] offers a highly sensitive cell line to detect both alphaviruses and flaviviruses.

Recent advances in diagnosis parallel those in virology in general with the application of radioimmune assay and the enzyme-linked immunosorbent assay [19] to serodiagnosis of arboviral encephalitis. In addition, with the advent of techniques which can differentiate IgM responses [20], an early presumptive diagnosis can be made in the U.S.A. on a single serum specimen since a predominantly IgM response with alphaviruses and flaviviruses in most cases means a recent infection.

I wish I could report to the clinical virologist that treatment or vaccines for arbovirus encephalitis were on the horizon. Both alphaviruses and flaviviruses are sensitive to interferon, but treatment attempts in monkey models were not successful. Monkeys with VEE infections had depressed serum levels of virus when treated with an interferon inducer, but the mortality was enhanced by the treatment for unknown reasons [21]. It is also difficult to conceive of vaccine application for most of the arbovirus encephalitides since the development cost would be tremendous and the application limited to focal population groups at sporadic intervals within unpredictable geographic areas. Conceivably, LaCrosse encephalitis might be an exception, because the disease is geographically delimited and appears mainly in children; the demonstration that this group of viruses has segmented RNA genomes and that reassortment takes place [22] could offer a basis for a recombinant vaccine analagous to that for influenza. A LaCrosse vaccine, even if it proved feasible, would probably not materialize for many years.

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